

What is claimed is:

1. A method of forcing recombination between polynucleotides, comprising:

5

(a) generating a single strand of a first polynucleotide;

(b) generating a single strand of a second polynucleotide, wherein said second polynucleotide is partially complementary to said first polynucleotide;

(c) fragmenting said single strand of said first polynucleotide to generate single stranded first polynucleotide fragments, and optionally isolating a size range of single stranded fragments;

(d) fragmenting said single strand of said second polynucleotide to generate single stranded second polynucleotide fragments, and optionally isolating a size range of single stranded fragments;

(e) annealing said single stranded first polynucleotide fragments with said single stranded second polynucleotide fragments; and

(f) extending said annealed polynucleotide fragments.

2. The method of claim 1, further comprising adding at least one additional single stranded polynucleotide partially complementary to said first or second polynucleotide.

3. The method of claim 1, further comprising

(g) denaturing said extended annealed
polynucleotide fragments; and

5

(h) repeating steps (e) and (f).

4. The method of claim 3, wherein steps (g) and (h)
are repeated one or more times, wherein a larger
10 polynucleotide is generated.

5. The method of any of claims 1 or 3, further
comprising the steps of:

15 (i) generating a single strand of a third
polynucleotide, wherein said third polynucleotide is the
exact complement of said first polynucleotide;

(j) generating a single strand of a fourth
20 polynucleotide, wherein said fourth polynucleotide is the
exact complement of said second polynucleotide;

(k) fragmenting said single strand of said third
polynucleotide to generate single stranded third
25 polynucleotide fragments, and optionally isolating a size
range of single stranded fragments;

(l) fragmenting said single strand of said fourth
polynucleotide to generate single stranded fourth
30 polynucleotide fragments, and optionally isolating a size
range of single stranded fragments;

(m) annealing said single stranded third
polynucleotide fragments with said single
35 stranded fourth polynucleotide fragments; and

(n) extending said annealed polynucleotide fragments.

5 6. The method of any of claims 1, 3 or 4, further comprising isolating a size range of double stranded polynucleotides.

10 7. The method of claim 6, further comprising adding primers and amplifying all or a portion of said extended annealed fragments.

15 8. The method of any of claims 1 through 4, further comprising adding primers and amplifying all or a portion of said extended annealed fragments.

20 9. The method of claim 3, further comprising selecting a recombinant polynucleotide to identify a recombinant nucleotide having a desired functional property.

25 10. The method of claim 3, further comprising screening a recombinant polynucleotide for a desired functional property.

 11. The method of claim 1, wherein fragmenting is performed enzymatically, chemically or physically.

30 12. The method of claim 1, wherein said single strand of said first polynucleotide and said second polynucleotide is generated by asymmetric PCR or single stranded nucleic acid vector.

0975049-04404

13. The method of any of claims 1 or 2, further comprising adding a first clamp to the 5' end of said first single stranded polynucleotide, and any other polynucleotides of the same sense, and a second clamp to the 5' end of said second polynucleotide, and any other polynucleotides of the complementary sense of said first single stranded polynucleotide.

14. A method of forcing recombination between polynucleotides, comprising:

(a) fragmenting a single stranded first polynucleotide and a single stranded second polynucleotide to generate single stranded first and second polynucleotide fragments, wherein said second polynucleotide is partially complementary to said first polynucleotide, and optionally isolating a size range of single stranded fragments;

(b) annealing said single stranded first polynucleotide fragments with said single stranded second polynucleotide fragments; and

(c) extending said annealed fragments.

15. The method of claim 14, further comprising adding at least one additional single stranded polynucleotide partially complementary to said first or second polynucleotide.

16. The method of claim 14, further comprising

(d) denaturing said extended annealed fragments; and

35

(e) repeating steps (b) and (c) one or more times, thereby generating one or more recombinant polynucleotides.

5 17. The method of claim 16, wherein steps (d) and (e) are repeated one or more times, wherein a full-length polynucleotide is generated.

18. The method of any of claims 14 or 16, further comprising the steps of:

10

(f) fragmenting a single stranded third polynucleotide, wherein said third polynucleotide is the exact complement of said first polynucleotide, and a single stranded fourth polynucleotide, wherein said
15 fourth polynucleotide is the exact complement of said second polynucleotide, to generate single stranded third and fourth polynucleotide fragments, and optionally isolating a size range of single stranded fragments;

20

(g) annealing said single stranded third polynucleotide fragments with said single stranded fourth polynucleotide fragments; and

25

(h) extending said annealed fragments.

19. The method of any of claims 14, 16, or 17, further comprising isolating a size range of double stranded polynucleotide.

30

20. The method of claim 19, further comprising adding primers and amplifying all or a portion of said extended annealed fragments.

21. The method of any of claims 14 through 17, further comprising adding primers and amplifying all or a portion of said extended annealed fragments.

5 22. The method of claim 16, further comprising selecting a recombinant polynucleotide to identify a recombinant nucleotide having a desired functional property.

10 23. The method of claim 16, further comprising screening a recombinant polynucleotide for a desired functional property.

15 24. The method of claim 14, wherein fragmenting is performed enzymatically, chemically or physically.

20 25. The method of claim 14, wherein said single strand of said first polynucleotide and said second polynucleotide are generated by asymmetric PCR or single stranded nucleic acid vector.

25 26. The method of claim 14, further comprising adding a first clamp to the 5' end of said first single stranded polynucleotide, and any other polynucleotides of the same sense, and a second clamp to the 5' end of said second polynucleotide, and any other polynucleotides of the complementary sense of said first single stranded polynucleotide.

30 27. A method of forcing recombination between polynucleotides, comprising:

(a) generating a single strand of a first polynucleotide;

(b) generating a single strand of a second polynucleotide, wherein said second polynucleotide is partially complementary to said first polynucleotide;

5 (c) annealing said single strands of said first and second polynucleotides;

(d) fragmenting said annealed polynucleotides to generate partially double stranded polynucleotide
10 fragments, and optionally isolating a size range of single stranded fragments;

(e) denaturing said partially double stranded polynucleotide fragments;

15 (f) annealing said denatured polynucleotide fragments; and

(g) extending said annealed polynucleotide fragments.

20 28. The method of claim 27, further comprising adding at least one additional single stranded polynucleotide partially complementary to said first or second polynucleotide.

25 29. The method of claim 27, further comprising

(h) denaturing said extended fragments;

30 (i) annealing said denatured extended fragments;
and

(j) extending said annealed extended fragments.

30. The method of claim 29, wherein steps (h) through (j) are repeated one or more times, wherein a full-length polynucleotide is generated.

5 31. The method of any of claims 27 or 29, further comprising the steps of:

(k) generating a single strand of a third polynucleotide, wherein said third polynucleotide is the
10 exact complement of said first polynucleotide;

(l) generating a single strand of a fourth polynucleotide, wherein said fourth polynucleotide is the exact complement of said second polynucleotide;

15 (m) annealing said single strands of said third and fourth polynucleotides;

(n) fragmenting said annealed polynucleotides to generate partially double stranded polynucleotide
20 fragments, and optionally isolating a size range of single stranded fragments;

(o) denaturing said partially double stranded polynucleotide fragments;

25 (p) annealing said denatured polynucleotide fragments; and

(q) extending said annealed polynucleotide
30 fragments.

32. The method of any of claims 25, 27 or 28, further comprising isolating a size range of double stranded polynucleotide.

33. The method of claim 32, further comprising adding primers and amplifying all or a portion of said extended fragments.

5 34. The method of any of claims 27 through 30, further comprising adding primers and amplifying all or a portion of said extended fragments.

10 35. The method of claim 30, further comprising selecting a recombinant polynucleotide to identify a recombinant nucleotide having a desired functional property.

15 36. The method of claim 29, further comprising screening a recombinant polynucleotide for a desired functional property.

20 37. The method of claim 27, wherein fragmenting is performed enzymatically, chemically or physically.

25 38. The method of claim 27, wherein said single strand of said first polynucleotide and said second polynucleotide is generated by asymmetric PCR or single stranded nucleic acid vector.

30 39. The method of claim 27 or 28, further comprising adding a first clamp to the 5' end of said first single stranded polynucleotide, and any other polynucleotides of the same sense, and a second clamp to the 5' end of said second polynucleotide, and any other polynucleotides of the complementary sense of said first single stranded polynucleotide.

35 40. A method of forcing recombination between polynucleotides, comprising:

(a) annealing a single stranded first polynucleotide with a single stranded second polynucleotide, wherein said second polynucleotide is
5 partially complementary to said first polynucleotide,

(b) fragmenting said annealed polynucleotides to generate partially double stranded polynucleotide fragments;
10

(c) denaturing said partially double stranded polynucleotide fragments;

(d) annealing said denatured polynucleotide fragments; and
15

(e) extending said annealed polynucleotide fragments.

20 41. The method of claim 40, further comprising adding at least one additional single stranded polynucleotide partially complementary to said first or second polynucleotide.

25 42. The method of claim 40, further comprising

(f) denaturing said extended polynucleotide;

(g) annealing said denatured extended
30 polynucleotide; and

(h) extending said annealed extended fragments.

0975040-043404

43. The method of claim 41, wherein steps (g) through (h) are repeated one or more times, wherein a full-length polynucleotide is generated.

5 44. The method of any of claims 40 or 42, further comprising the steps of:

10 (i) annealing a single stranded third polynucleotide with a single stranded fourth polynucleotide, wherein said third polynucleotide is the exact complement of said first polynucleotide and said fourth polynucleotide is the exact complement of said second polynucleotide;

15 (j) fragmenting said annealed polynucleotides to generate partially double stranded polynucleotide fragments;

20 (k) denaturing said partially double stranded polynucleotide fragments;

 (1) annealing said denatured polynucleotide fragments; and

25 (m) extending said annealed polynucleotide fragments.

30 45. The method of claims 40, or 42 through 43, further comprising isolating a size range of double stranded polynucleotide.

 46. The method of claim 45, further comprising adding primers and amplifying all or a portion of said extended fragments.

47. The method of any of claims 40 through 43, further comprising adding primers and amplifying all or a portion of said extended fragments.

5 48. The method of claim 47, further comprising selecting a recombinant polynucleotide having a desired functional property.

10 49. The method of claim 47, further comprising screening a recombinant polynucleotide for a desired functional property.

15 50. The method of claim 40, wherein fragmenting is performed enzymatically, chemically or physically.

20 51. The method of claim 40, wherein said single strand of said first polynucleotide and said second polynucleotide is generated by asymmetric PCR or single stranded nucleic acid vector.

25 52. The method of claim 40 or 41, further comprising adding a first clamp to the 5' end of said first single stranded polynucleotide, and any other polynucleotides of the same sense, and a second clamp to the 5' end of said second polynucleotide, and any other polynucleotides of the complementary sense of said first single stranded polynucleotide.

30 53. A method of forcing recombination between polynucleotides, comprising:

(a) generating a single strand of a first polynucleotide;

(b) generating a single strand of a second polynucleotide, wherein said second polynucleotide is partially complementary to said first polynucleotide;

5 (c) generating a single strand of a third polynucleotide;

(d) fragmenting said single strand of said first polynucleotide to generate single stranded first
10 polynucleotide fragments, and optionally isolating a size range of single stranded fragments;

(e) fragmenting said single strand of said second polynucleotide to generate single stranded second
15 polynucleotide fragments, and optionally isolating a size range of single stranded fragments;

(f) fragmenting said single strand of said third polynucleotide to generate single stranded third
20 polynucleotide fragments, and optionally isolating a size range of single stranded fragments;

(g) annealing said single stranded first polynucleotide fragments with said single stranded second
25 polynucleotide fragments;

(h) extending said annealed polynucleotide

(i) denaturing said extended annealed
30 polynucleotide fragments;

(j) annealing said single stranded third polynucleotide fragments with said extended, annealed, and denatured polynucleotide fragments from step (g); and

(k) extending said annealed polynucleotide fragments.

54. The method of claim 53 wherein said single stranded third polynucleotide has greater sequence
5 homology to said single stranded first polynucleotide than to said single stranded second polynucleotide.

55. The method of claim 53 wherein said single stranded third polynucleotide has greater sequence
10 homology to said single stranded second polynucleotide than to said single stranded first polynucleotide.

56. The method of claim 53, further comprising adding at least one additional single stranded
15 polynucleotide partially complementary to said first, second, or third polynucleotide.

57. The method of claim 53, further comprising:

20 (l) denaturing said extended annealed polynucleotide fragments; and

(m) repeating steps (j), and (k).

25 58. The method of claim 57, wherein steps (l) and (m) are repeated one or more times, wherein a larger polynucleotide is generated.

59. The method of claim 53, further comprising:

30

(n) generating a single strand of a fourth polynucleotide, wherein said fourth polynucleotide is the exact complement of said first polynucleotide;

(o) generating a single strand of a fifth polynucleotide, wherein said fifth polynucleotide is the exact complement of said second polynucleotide;

5 (p) generating a single strand of a sixth polynucleotide, wherein said sixth polynucleotide is the exact complement of said third polynucleotide;

10 (q) fragmenting said single strand of said fourth polynucleotide to generate single stranded fourth polynucleotide fragments, and optionally isolating a size range of single stranded fragments;

15 (r) fragmenting said single strand of said fifth polynucleotide to generate single stranded fifth polynucleotide fragments, and optionally isolating a size range of single stranded fragments;

20 (s) fragmenting said single strand of said sixth polynucleotide to generate single stranded sixth polynucleotide fragments, and optionally isolating a size range of single stranded fragments;

25 (t) annealing said single stranded fourth polynucleotide fragments with said single stranded fifth polynucleotide fragments;

(u) extending said annealed polynucleotide fragments;

30

(v) denaturing said extended annealed polynucleotide fragments;

(w) annealing said single stranded sixth polynucleotide fragments with said extended, annealed, and denatured polynucleotide fragments from step (t); and

5 (x) extending said annealed polynucleotide fragments.

60. The method of any of claims 53, 57 or 58, further comprising isolating a size range of double stranded polynucleotides after either step (f), step (k),
10 step (l) or step (m).

61. The method of claim 60, further comprising adding primers and amplifying all or a portion of said extended annealed fragments.
15

62. The method of any of claims 53 through 58, further comprising adding primers and amplifying all or a portion of said extended annealed fragments.

20 63. The method of claim 57, further comprising selecting a recombinant polynucleotide to identify a recombinant nucleotide having a desired functional property.

25 64. The method of claim 57, further comprising screening a recombinant polynucleotide for a desired functional property.

30 65. The method of claim 53, wherein fragmenting is performed enzymatically, chemically or physically.

66. The method of claim 53, wherein said single strand of said first polynucleotide and said second polynucleotide is generated by asymmetric PCR or single
35 stranded nucleic acid vector.

67. The method of claim 53 or 56, further comprising adding a first clamp to the 5' end of said first single stranded polynucleotide, and any other
5 polynucleotides of the same sense, and a second clamp to the 5' end of said second polynucleotide, and any other polynucleotides of the complementary sense of said first single stranded polynucleotide.

10 68. A method of forcing recombination between polynucleotides, comprising:

(a) generating a single strand of a first polynucleotide;
15

(b) generating a single strand of a second polynucleotide, which is partially complementary to said first polynucleotide;

20 (c) generating a fragmented single strand of a third polynucleotide, which is partially complementary to said first polynucleotide or second polynucleotide;

25 (d) annealing said single strands of said first and second polynucleotides;

(e) fragmenting said annealed polynucleotides to generate partially double stranded polynucleotide fragments;
30

(f) denaturing said partially double stranded polynucleotide fragments;

35 (g) annealing said denatured polynucleotide fragments;

(h) extending said annealed polynucleotide fragments;

5 (i) denaturing said partially double stranded polynucleotide fragments;

(j) adding said fragmented single strand of said third polynucleotide to denatured partially double
10 stranded polynucleotide fragments;

(k) annealing said fragmented single strand of said third polynucleotide either to extended first polynucleotide fragments or to extended second
15 polynucleotide fragments;

(l) denaturing said partially double stranded polynucleotide fragments;

20 (m) annealing said denatured polynucleotide fragments; and

(n) extending said annealed polynucleotide fragments.
25

69. The method of claim 68, further comprising adding at least one additional single stranded polynucleotide partially complementary to said first or second polynucleotide.
30

70. The method of claim 68, further comprising:
(o) denaturing said extended fragments;

(p) annealing said denatured extended fragments; and
35

(q) extending said annealed extended fragments.

71. The method of claim 73, wherein steps (o) through (q) are repeated one or more times, wherein a full-length polynucleotide is generated.

72. The method of any of claims 70 or 71, further comprising:

10 (r) generating a single strand of a fourth polynucleotide, wherein said fourth polynucleotide is the exact complement of said first polynucleotide;

(s) generating a single strand of a fifth polynucleotide, wherein said fifth polynucleotide is the exact complement of said second polynucleotide;

(t) generating a fragmented single strand of a sixth polynucleotide, wherein said sixth polynucleotide is the exact complement of said third polynucleotide;

(u) annealing said single strands of said fourth and fifth polynucleotides;

25 (v) fragmenting said annealed polynucleotides to generate partially double stranded polynucleotide fragments;

(w) denaturing said partially double stranded polynucleotide fragments;

(x) annealing said denatured polynucleotide fragments;

(y) extending said annealed polynucleotide fragments;

(z) denaturing said partially double stranded polynucleotide fragments;

(aa) adding said fragmented single strand of said sixth polynucleotide to denatured partially double stranded polynucleotide fragments;

10

(bb) annealing said fragmented single strand of said sixth polynucleotide either to extended fourth polynucleotide fragments or to extended fifth polynucleotide fragments;

15

(cc) denaturing said partially double stranded polynucleotide fragments;

(dd) annealing said denatured polynucleotide fragments; and

20

(ee) extending said annealed polynucleotide fragments.

73. The method of any of claims 68, 70 or 71, further comprising isolating a size range of double stranded polynucleotide after step (e), step (h), step (m), or step (q).

25

74. The method of claim 73, further comprising adding primers and amplifying all or a portion of said extended fragments.

30

75. The method of any of claims 68 through 71, further comprising adding primers and amplifying all or a portion of said extended fragments.

5 76. The method of claim 68 or 70, further comprising selecting a recombinant polynucleotide to identify a recombinant nucleotide having a desired functional property.

10 77. The method of claim 68 or 70, further comprising screening a recombinant polynucleotide for a desired functional property.

15 78. The method of claim 68, wherein fragmenting is performed enzymatically, chemically or physically.

20 79. The method of claim 68, wherein said single strand of said first polynucleotide and said second polynucleotide is generated by asymmetric PCR or single stranded nucleic acid vector.

25 80. The method of any of claims 68 or 71, further comprising adding a first clamp to the 5' end of said first single stranded polynucleotide, and any other polynucleotides of the same sense, and a second clamp to the 5' end of said second polynucleotide, and any other polynucleotides of the complementary sense of said first single stranded polynucleotide.

30 81. The method of claim 68 further comprising extending said partially double stranded polynucleotide fragments after step (e).

35 82. A method of forcing recombination between polynucleotides, comprising:

(a) generating a single strand of a first polynucleotide;

5 (b) generating a single strand of a second polynucleotide, which is partially complementary to said first polynucleotide;

10 (c) generating a single strand of a third polynucleotide, which is partially complementary to said first polynucleotide or second polynucleotide;

15 (d) annealing said single strands of said first and second polynucleotides;

(e) fragmenting said annealed polynucleotides to generate partially double stranded polynucleotide fragments;

20 (f) denaturing said partially double stranded polynucleotide fragments;

25 (g) annealing said denatured polynucleotide fragments;

(h) extending said annealed polynucleotide fragments;

30 (i) denaturing said partially double stranded polynucleotide fragments;

(j) adding said single strand of said third polynucleotide to denatured partially double stranded polynucleotide fragments;

(k) annealing said single strand of said third polynucleotide either to extended first polynucleotide fragments or to extended second polynucleotide fragments;

5 (l) fragmenting said annealed polynucleotides to generate partially double stranded polynucleotide fragments, and optionally isolating a size range of single stranded fragments;

(m) denaturing said partially double stranded
10 polynucleotide fragments;

(n) annealing said denatured polynucleotide fragments; and

15 (o) extending said annealed polynucleotide fragments.

83. The method of claim 82, further comprising adding at least one additional single stranded
20 polynucleotide partially complementary to said first or second polynucleotide.

84. The method of claim 82, further comprising:

25 (p) denaturing said extended fragments;

(q) annealing said denatured extended fragments; and

(r) extending said annealed extended fragments.
30

85. The method of claim 83, wherein steps (p) through (r) are repeated one or more times, wherein a full-length polynucleotide is generated.

86. The method of any of claims 82 or 84, further comprising

(s) generating a single strand of a fourth
5 polynucleotide, wherein said fourth polynucleotide is the exact complement of said first polynucleotide;

(t) generating a single strand of a fifth
10 polynucleotide, wherein said fifth polynucleotide is the exact complement of said second polynucleotide;

(u) generating a single strand of a sixth
15 polynucleotide, wherein said sixth polynucleotide is the exact complement of said third polynucleotide;

(v) annealing said single strands of said fourth and fifth polynucleotides;

20 (w) fragmenting said annealed polynucleotides to generate partially double stranded polynucleotide fragments;

(x) denaturing said partially double stranded
25 polynucleotide fragments;

(y) annealing said denatured polynucleotide fragments;

30 (z) extending said annealed polynucleotide fragments;

(aa) denaturing said partially double stranded
35 polynucleotide fragments;

(bb) adding said single strand of said sixth polynucleotide to denatured partially double stranded polynucleotide fragments;

5 (cc) annealing said single strand of said sixth polynucleotide either to extended fourth polynucleotide fragments or to extended fifth polynucleotide fragments;

(dd) fragmenting said annealed polynucleotides to
10 generate partially double stranded polynucleotide fragments, and optionally isolating a size range of single stranded fragments;

(ee) denaturing said partially double stranded
15 polynucleotide fragments;

(ff) annealing said denatured polynucleotide fragments; and

(gg) extending said annealed polynucleotide
20 fragments.

87. The method of any of claims 82, 84 or 85, further comprising isolating a size range of double stranded polynucleotide after step (e), step (h), step
25 (m) or (r).

88. The method of claim 87, further comprising adding primers and amplifying all or a portion of said extended fragments.

30 89. The method of any of claims 82 through 85, further comprising adding primers and amplifying all or a portion of said extended fragments.

90. The method of claim 82 or 84, further comprising selecting a recombinant polynucleotide to identify a recombinant nucleotide having a desired functional property.

5

91. The method of claim 82 or 84, further comprising screening a recombinant polynucleotide for a desired functional property.

10

92. The method of claim 82, wherein fragmenting is performed enzymatically, chemically or physically.

15

93. The method of claim 82, wherein said single strand of said first polynucleotide and said second polynucleotide is generated by asymmetric PCR or single stranded nucleic acid vector.

20

94. The method of claim 82 or 83, further comprising adding a first clamp to the 5' end of said first single stranded polynucleotide, and any other polynucleotides of the same sense, and a second clamp to the 5' end of said second polynucleotide, and any other polynucleotides of the complementary sense of said first single stranded polynucleotide.

25

95. The method of claim 82 further comprising extending said partially double stranded polynucleotide fragments after step (e).